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14. ABSTRACT

The goal of this project is to determine if resveratrol can enhance the anti-proliferative response of Zinc (Zn) against prostate cancer (PCa) via increasing ZIP1 mediated Zn transport. Our proposed in vivo pre-clinical experiments in PTEN mouse model of PCa are currently under progress. We are currently breeding the prostate specific PTEN knockout mice and have also started experiments with another transgenic model (TRAMP) model, due to their ready availability. Further, in additional experiments we have found that that ZIP1 protein is markedly downregulated in human PCa tissue and cell lines compared to normal prostate tissue and normal RWPE-1 cells. In cell culture, resveratrol- Zn combination was found to result in a marked increase in ZIP1 mRNA and protein in PCa cells. In addition, resveratrol- Zn resulted in a superior anti-proliferative response in PCa cells, compared to either of the agent alone. Although, the data from the completed in vivo trial will be available after the completion of studies, our initial observations coupled with in vitro data suggests that our hypothesis may be valid and resveratrol- Zn combination may have superior anti-proliferative response against PCa.

15. SUBJECT TERMS
prostate cancer, zinc transporters, zinc, resveratrol, chemoprevention

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INTRODUCTION:

Following specific aims were proposed in the funded PC110626 post-doctoral award application:

1. To determine if a combination of resveratrol and Zn imparts superior chemopreventive and/or therapeutic response against PCa in PTEN knockout mice.
2. To determine the molecular mechanism(s) of resveratrol-Zn combinatorial action.

The research proposed in this application was aimed at identifying novel means and approaches for the management of PCa, which next only to skin cancer, is the most common cancer in American men (1). In humans, high levels of Zn are found in normal prostate glands. However, Zn levels are significantly lower in PCa. The loss of the unique ability of the prostate to retain normal intracellular levels of Zn has been shown to be associated with Zn transporter ZIP1, and established as an important factor in the development and progression of PCa (2). Increased levels of intracellular Zn by Zn supplementation have been shown to decrease growth of PCa cells. However, to increase the intracellular Zn accumulation by high concentration Zn supplementation is reported to impose a lot of adverse health effects. Here, we proposed to utilize a strategy of combining Zn with resveratrol to enhance the bioaccumulation of Zn in prostatic tissue which may lead to a blockade of the mechanisms contributing to prostate carcinogenesis.

PROGRESS REPORT:

In our DOD funded PC110626 project, we proposed to test the hypothesis that ***resveratrol when combined with Zn will enhance its bioaccumulation, via increasing Zn-transporter ZIP1 in prostate, to impart a significantly superior chemopreventive and therapeutic response against PCa.*** Overall we are making significant progress as described below.

Proposed in vivo studies: Our plan was to conduct a pre-clinical trial in the PTEN knockout mouse model of PCa. We are currently breeding the mice to obtain enough number for our experiments. We are crossing $PTEN^{loxP/loxP}$ mice with ARR2 probasin-cre transgenic line PB-cre4, wherein the Cre recombinase is under the control of a modified rat prostate-specific probasin promoter. Cross breeding of the F1 generation offsprings leads to a homozygous deletion of PTEN in the F2 generation. PTEN deletion in these mice is being confirmed by PCR using tail DNA. However, the breeding process is taking longer than we had anticipated. Therefore, in order to continue with our experiments, while the breeding of PTEN mice is ongoing, we have started the in vivo pre-clinical trial for resveratrol-Zn combination in Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model. TRAMP mice are considered an excellent animal model of human PCa and a clinically relevant model for PCa biology and therapeutic studies. In addition, our lab has an active TRAMP mouse breeding colony from which we can easily obtain mice.

These mice are suitable for our study because a recent study has shown that the TRAMP model contains hallmark characteristics of PCa (3) *i.e.* i) decreased intracellular Zn level, ii) decreased citrate level, and iii) down regulation of ZIP1 Zn transporter in prostate malignancy. TRAMP mice also recapitulate key features of early as well as advanced stages of human PCa. These mice show epithelial hyperplasia by 8 weeks of age, progression to prostatic intraepithelial neoplasia (PIN) by 18 weeks of age, and after 28 weeks of age, display lymphatic metastases (4, 5) (Figure 4).

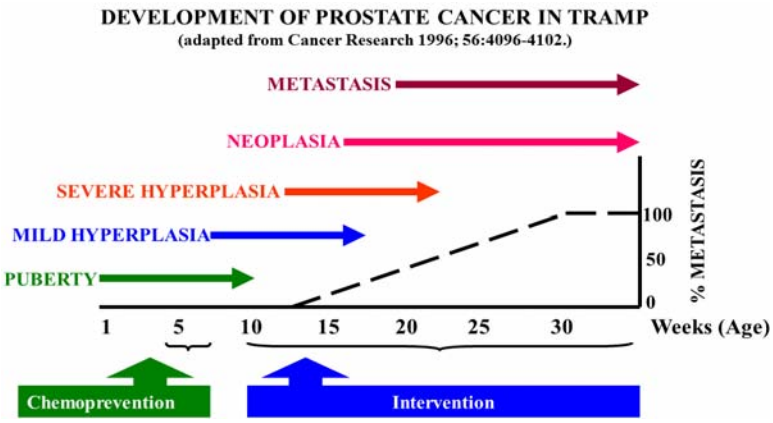

Figure 4: PCa progression in TRAMP mice (5).

The design of our preclinical trial is as follows:

Prevention Trial: Start at 4 weeks, euthanize at 28 weeks.

Intervention Trial: Start at 14 weeks, euthanize at 28 weeks.

We have the following six experimental groups (12 animals/group) in our protocols:

1. Control (Vehicle alone)
2. ZnSO4.7H2O (15 ppm in drinking water) that corresponds to 7.5 mg/kg b. wt.
3. ZnSO4.7H2O (30 ppm in drinking water) that corresponds to 15 mg/kg b. wt.
4. Resveratrol (600 mg/kg diet) that corresponds to 100 mg/kg b. wt.
5. Resveratrol (600 mg/kg diet) + ZnSO4.7H2O (15 ppm in drinking water)
6. Resveratrol (600 mg/kg diet) + ZnSO4.7H2O (30 ppm in drinking water)

Our original plan was to administer the test agents via oral gavage. However, we modified our experimental strategy in light of recent research developments. We decided to give resveratrol in fortified diet and Zn in drinking water to allow for less stress to the animals, as well as a better way to decrease potential drug interactions. We are using ZnSO4.7H2O as the source of Zn, and its dose was selected on basis of our preliminary studies and one recent report where 30 ppm Zn was considered as optimal Zn dose (6). Since the required numbers of animals for each group or the entire experiment are difficult to obtain at one time, we are performing the studies with available mice and continued to add mice as and when available, until each group reaches the required number. We have completed the control and resveratrol treated TRAMP mice groups. So far, our data seems to suggest that resveratrol alone has protective effects against PCa development. The other groups are still ongoing, and it is difficult to make any prediction at this time.

As mentioned in our statement of work, we are terminating the experiment when each mouse reaches 28 weeks of age; at which point tumor characteristics are being assessed which involve tumor picture, size and tumor wet weight. Simultaneously, mouse serum is being collected at every fourth week and after sacrifice to evaluate the levels of IGF-1 and IGFBP-3.

Additional in vitro and ex vivo experiments: In addition to the proposed in vivo worked, we conducted in vitro and ex vivo studies to test our hypothesis. A brief description of these experiments is provided below:

In order to assess the anti-proliferative effect of resveratrol-Zn combination in PCa, we performed in vitro experiments in human PCa cells. We found that resveratrol-Zn combination imparts superior anti-proliferative response in human PCa cells as compared to either of the agents alone. Initially, this study was performed on androgen-responsive 22Rv1 and androgen unresponsive DU145 cells, later it was also confirmed on PC3, LNCaP and C4-2 prostate cancer cells. Further, resveratrol-Zn combination imparts superior inhibition of clonogenic survival of human PCa cells (Figure 1A) and induction of apoptosis (Figure 1B). All the in vitro studies were performed using six experimental groups; (1) control - dmso, (2) Z50 – Zn 50 µM, (3) Z100 – Zn 100 µM, (4) RSV25 – resveratrol 25 µM, (5) RZ50 - Zn 50 µM + resveratrol 25 µM, (6) RZ100 - Zn 100 µM + resveratrol 25 µM.

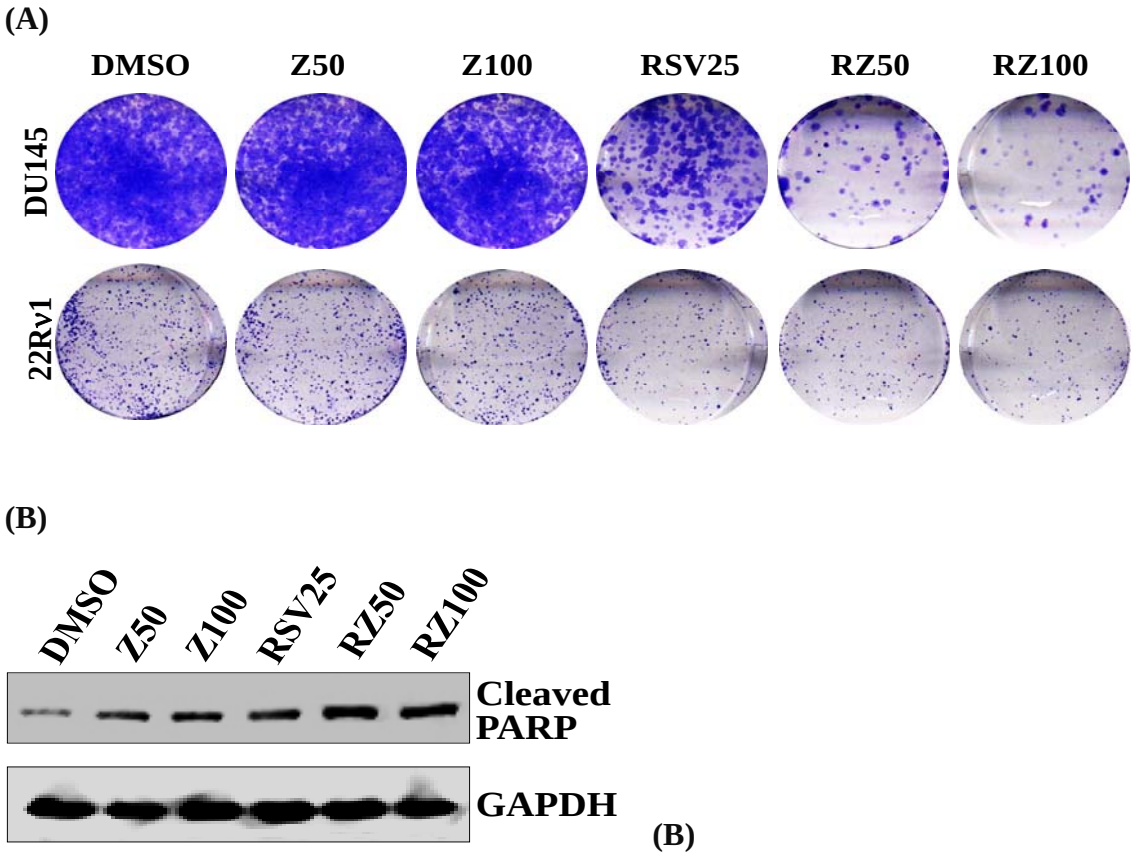

Figure 1: Resveratrol-Zn combination inhibits clonogenic survival of human DU145 *and* 22Rv1 *cells (A); and enhances apoptotic response in DU145 cells (B).*

Next, employing a prostate carcinoma tissue microarray (US Biomax, Inc.), we assessed the status of Zn transporter ZIP1 in human PCa tissues. We found that ZIP1 is significantly downregulated in human prostatic carcinoma compared to normal prostate tissues (Figure 2A).

Further, we employed a panel of human PCa cells in order to assess the status of ZIP1 at protein as well as on mRNA levels. Our data further confirmed ZIP1 downregulation in comparison to immortalized normal prostate epithelial cells RWPE-1(Figure 2B and 2C). We are currently validating these data in additional experiments for a statistical analysis.

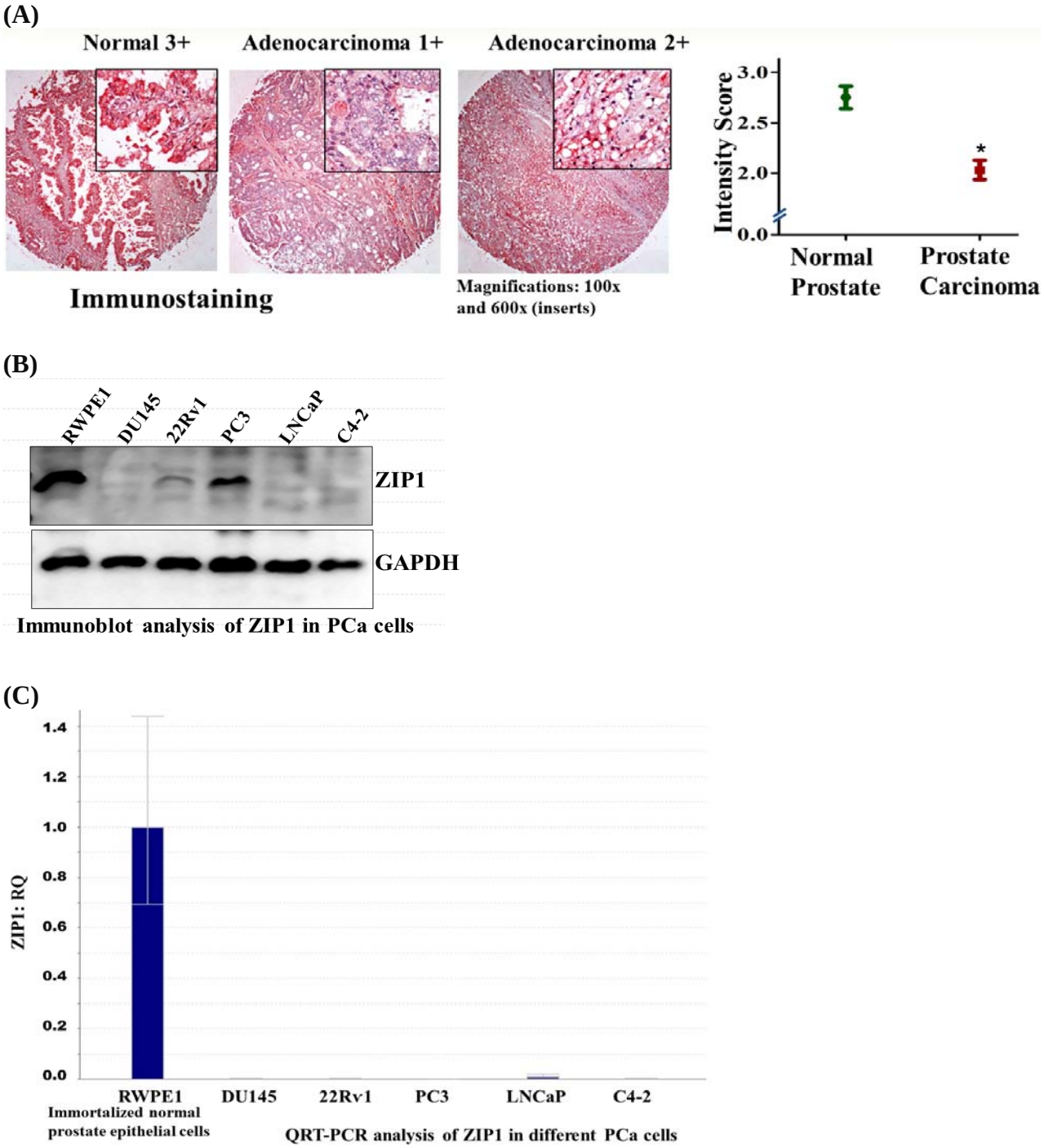

Figure 2: ZIP1 is downregulated in human PCa as evident from ZIP1 immunostaining of human PCa tissues (A), ZIP1 immunoblot data (B) and QRT-PCR analysis of ZIP1 in PCa cells (C).

In another experiment, we evaluated the effect of resveratrol-Zn combination on ZIP1 expression on DU145 and 22Rv1 cells. We found a significant increase in ZIP1 at both levels mRNA as well as protein in resveratrol treated groups, especially in the RZ100 group (Figure 3A and B).

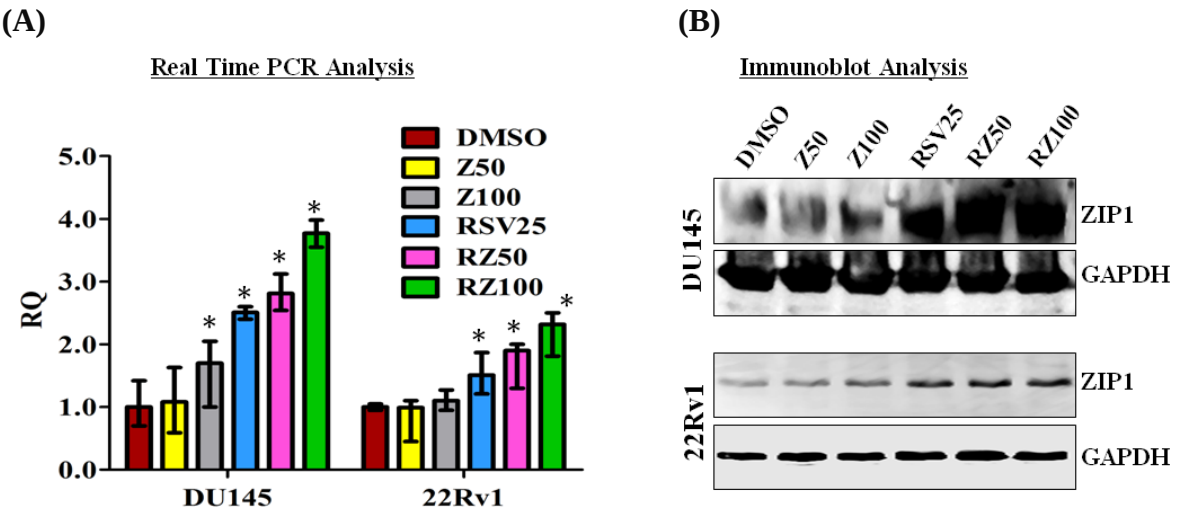

Figure 3: Resveratrol-Zn combination increases ZIP1 level in human PCa cells as evident from QRT-PCR analysis (A) and Immunoblot data (B).

At present, we are conducting experiment to assess intracellular Zn levels in PCa cells following resveratrol-Zn treatment. The Zn content in the prostate gland is mainly regulated by two gene families. ZnT family transporters reduce intracellular Zn while ZIP family transporters increase intracellular Zn. ZIP1 was the first Zn transporter to be connected with PCa progression and may be the major regulator of Zn transport in this organ. However, recent studies have revealed ZIP2, ZIP3 and ZIP4 modulations in malignant prostate cells. Therefore, we are also checking the status of other zinc transporters in PCa and with effect of resveratrol-Zn combination.

KEY RESEARCH ACCOMPLISHMENTS:

- We have been able to complete two groups (control as well as resveratrol group) in our ongoing clinical trial with TRAMP mice, and tumor data is encouraging. For other four experimental groups, studies are ongoing.

- We are currently breeding PTEN mice in our animal facility and very soon we should be able to get F2 progeny of PTEN mice for our experiments.

- Additional ex vivo and in vitro data suggest that ZIP1 is downregulated in PCa tissues and cells; and resveratrol-Zn combination increases ZIP1 Zn transporter and imparts a superior anti-proliferative response (inhibition of cell growth/viability, clonogenic survival and induction of apoptosis) in PCa cells. We are currently analyzing the data in detail and plan to submit a manuscript (containing our findings) in the next 2-3 months.

REPORTABLE OUTCOMES:

We have been able to publish one paper discussing the prospects of resveratrol in combination for cancer management. The citations of this publication are as follows:

- Singh CK, George J, Ahmad N: Resveratrol-based combinatorial strategies for cancer management. *Annals of the New York Academy of Science* [In Press].

The financial support from DOD has been acknowledged in this publication. A copy of the manuscript is attached in the Appendix.

CONCLUSION:

The poor bioaccumulation of Zn in prostate malignancy is viewed as a major obstacle towards Zn based approach for PCa management. We had proposed a mechanistically-driven strategy to enhance the bioaccumulation of Zn via modulating Zn transporter protein by resveratrol-Zn combination. Our in vivo preclinical trials are currently ongoing, so it is difficult to make any conclusions at this time. However, our in vitro data on different PCa cell lines and in vivo data of resveratrol treated TRAMP mice indicate a promising outcome. After completion of in vivo prevention and intervention trial (in both TRAMP and PTEN mice), we should be able to obtain useful information regarding role of resveratrol-Zn combination for PCa management.

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APPENDICES:

Article in Press is enclosed.

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Resveratrol-based combinatorial strategies for cancer management

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In recent years, *combination chemoprevention* is being increasingly appreciated and investigated as a viable and effective strategy for cancer management. A plethora of evidence suggests that a combination of agents may afford synergistic (or additive) advantage for cancer management by multiple means, such as by (1) enhancing the bioavailability of chemopreventive agents, (2) modifying different molecular targets, and (3) lowering the effective dose of agents/drug to be used for cancer management. Resveratrol has been shown to afford chemopreventive as well as therapeutic effects against certain cancers. Recent studies are suggesting that resveratrol may be very useful when given in combination with other agents. The two major advantages of using resveratrol in combination with other agents are (1) synergistically or additively enhancing the efficacy against cancer, and (2) limiting the toxicity and side effects of existing therapies. However, concerted and multidisciplinary efforts are needed to identify the most optimal combinatorial strategies.

Keywords: resveratrol; cancer; chemoprevention; combination chemoprevention

Introduction

Amassed research has suggested that a number of naturally occurring agents, including those present in human diet, may be useful against a variety of diseases including cancer. However, based on the recent literature, it is becoming increasingly clear that a single-agent approach is probably less likely to be very effective in the management of diseases, including cancer. In fact, a combinatorial approach relying on a cocktail of drugs, rather than a single drug, has been in practice for disease management for a long time. As pointed out by Dr. Michael Sporn and also suggested by recent research, *combination chemoprevention* may be a more practical approach for cancer management.[1] In classical terms, chemoprevention is defined as a strategy to reduce the risk, or delay the development or recurrence, of cancer via drugs, vitamins, or other agents. However, recent studies have suggested the usefulness of a number of chemopreventive agents in therapeutic settings. Therefore, the definition of chemoprevention seems to have expanded to include the delay or even reversal of the process of carcinogenesis. It appears that combinatorial chemopreventive approaches could be effective in prevention as well as treatment of cancer. The effective combination chemopreventive approaches can make use of (1) a combination of multiple agents based on molecular targets, (2) a combination of existing drugs with chemopreventive agents, in adjuvant settings, and/or (3) a combination of agents, drugs, and life style modifications.

Resveratrol, an antioxidant present in red grapes, red wine, and a variety of other dietary sources, has been shown to possess many beneficial biological properties, including cancer chemopreventive effects. A plethora of studies, especially in the past 15 years, have shown the cancer preventive and therapeutic potential of resveratrol in a variety of *in vitro* and *in vivo* models. In the recent past, resveratrol has arguably become the agent that holds the most fascination among researchers, the news media, and the general public. Some recent studies have also evaluated the combinatorial effects of resveratrol with other naturally occurring and chemotherapeutic agents, suggesting that resveratrol can improve

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the efficacy of other agents.[2–8] Indeed, the strategy of using resveratrol in combination with other agents, particularly with chemotherapeutic modalities, holds a clinical promise in cancer management. However, evidence-based scientific evaluations in appropriate models are needed to show the efficacy of resveratrol in combination with other agents. This review provides a discussion and perspective on the potential of resveratrol-based combinatorial strategies for cancer management.

Resveratrol amid many of nature's gifts

Resveratrol, chemically known as 3,5,4′-trihydroxy-trans-stilbene, is a strong antioxidant that has been identified in over 70 plant species, including grape skin, raspberries, blueberries, mulberries, Scots pine, Eastern white pine, and knotweed. Resveratrol is a phytoalexin, synthesized *de novo* by plants during environmental stress and pathogenic invasion, thereby acting as a natural inhibitor of cell proliferation.[9] The use of resveratrol for health benefits can be traced back to several ancient medicine systems. For example, resveratrol has been a component of "Darakchasava," an ancient Ayurvedic herbal formulation.[10] However, resveratrol was first isolated by Michio Takaoka from the roots of *Veratrum grandiflorum* (white hellebore) in 1940 (reviewed in Timmers *et al.*).[11] In 1963, he extracted resveratrol from the roots of the plant *Polygonum cuspidatum* (Japanese knotweed). At present, most of the commercially available resveratrol is isolated from *Polygonum cuspidatum* using high-speed counter-current chromatography.[12] The popularity of resveratrol started rising in 1992 when its occurrence was noticed in red wine and it was linked to "French Paradox," the apparently paradoxical epidemiological observation that the French population possesses a lower risk of coronary heart disease, despite consuming a diet rich in saturated fats.[11] Following this, scientific research on resveratrol surged at an astronomical pace. Although resveratrol exists in both *cis*- and *trans*-stereoisomeric forms, the commercially available resveratrol is mainly the *trans*-form and that has been most extensively studied. Because of its strong antioxidant properties, resveratrol is being extensively studied in a variety of oxidative stress-associated diseases. A number of studies have shown the benefits of resveratrol against a variety of diseases and conditions including heart disease, neurological disorders, metabolic disorders, and degenerative conditions. Resveratrol has also been shown to improve immune function and mimic the life-lengthening effects of calorie restriction without dieting. The cancer chemopreventive properties of resveratrol were first appreciated in 1997, when Jang *et al.* found that resveratrol possesses chemopreventive activity against all the three major stages of carcinogenesis (i.e., initiation, promotion, and progression).[13] This was followed by an extensive effort of researchers to determine the cancer chemopreventive and therapeutic effects of resveratrol in a wide range of models.

Resveratrol for cancer management

Popularity of resveratrol in cancer chemoprevention research could be appreciated from its continuously growing records in PubMed as well as the clinical trial databases. Based on published studies, there is sufficient evidence that resveratrol possesses promise in chemoprevention of several cancer types. Below, we have provided a very brief description on selected published studies suggesting chemopreventive/antiproliferative effects of resveratrol against some cancer types.

Several studies have suggested that resveratrol could be useful against prostate cancer, which is a major neoplasm of males and represents an ideal candidate disease for chemoprevention due to its long latency and identifiable preneoplastic lesions. Resveratrol has been demonstrated to impart chemopreventive effects in relevant animal models of prostate cancer. Harper *et al.* have shown that resveratrol reduced the incidences of poorly differentiated prostatic adenocarcinoma by several folds in the transgenic adenocarcinoma of mouse prostate (TRAMP) model.[14] Seeni *et al.* have demonstrated that resveratrol suppresses prostate cancer growth in the transgenic rat for adenocarcinoma of prostate (TRAP) model.[15]

The first evidence regarding the possible skin cancer chemopreventive efficacy of resveratrol comes from the study by Jang *et al.* that demonstrated chemopreventive effects of resveratrol in the classic chemical carcinogenesis model.[13] Since ultraviolet (UV) light is believed to be the major cause of skin cancer, in a series of studies from our laboratory, we demonstrated the protective potential of resveratrol against UV-mediated damage in skin (reviewed in Ndiaye *et al.*).[16] In an important study, employing a UVB initiation–promotion protocol, we

demonstrated that the topical application of resveratrol resulted in a significant inhibition in skin tumor incidence as well as delay in the onset of tumorigenesis in an SKH-1 hairless mouse model.[17] Following this study, several reports demonstrated the protective efficacy of resveratrol against skin cancer (reviewed in Ref. 16). In addition, resveratrol has also been shown to be effective in syngeneic melanoma mouse models.[18] Similarly, a number of studies have demonstrated the potential efficacy of resveratrol against breast cancer,[19] gastric cancer,[20] colorectal cancers,[21–23] and other cancer types such as cancers of lung, liver, pancreas, and bladder.[24–27] Thus, resveratrol has been extensively studied for cancer chemoprevention and may have the potential to become an ideal agent for cancer management. Further, resveratrol does not seem to have toxicity and has been shown to be reasonably well tolerated at doses of up to 5 g/day in healthy subjects without any side effects.[28] However, the effective dose of resveratrol depends on disease and subject context, and still needs to be investigated.

Combination chemoprevention from ancient to modern time

The concept of combination chemoprevention is not a new idea. Most of the world's ancient medicine systems seem to have relied on multiple agents to try to target many symptoms at the same time. *Ayurveda* (meaning "the science of long life" in Sanskrit), or ayurvedic medicine, an approximately 5000-year-old system of traditional medicine native to the Indian subcontinent, often uses a combination of herbs and agents for disease management. Ayurveda is still in practice in the Indian subcontinent for management of diseases including cancer.[29] There is an extensive list of herbs that are used, often in combinations, in the Ayurvedic management of cancer. Some of these, which have been tested and supported by modern research to have antiproliferative efficacy, include *Curcuma longa* (turmeric), *Aloe vera* (aloe), *Allium sativum* (garlic), *Abrus precatorium* (coral bead vine), *Boswellia serrata* (Indian olibanum), *Plumbago zeylanica* (leadwort), and *Vinca rosea* (periwinkle).[29] Interestingly, the herbal Ayurvedic tonic formulation Darakchasava, which is used for good health, has been shown to contain resveratrol and pterostilbene.[10] Similarly, the traditional Chinese medicine system, which also has a more than 5000-year-old history, is also based on a cocktail approach. Traditional Chinese herbal cocktails are often used as complementary medicine approaches to manage diseases, including in cancer to diminish the side effects and/or tumor resistance to chemotherapy/radiotherapy.[30] Interestingly, a cocktail of Chinese herbs (containing spreading hedyotis herb, barbed skullcap herb, ma-yuen Job's tears seed, *Ganoderma lucidum*, and Chinese hawthorn fruit), in conjunction with chemotherapy and radiation therapy, was shown to have favorable clinical outcome in pancreatic cancer patients with liver metastases.[31] Nature also seems to support a combinatorial approach, since our food is believed to be a conglomeration of numerous beneficial ingredients. Based on emerging scientific evidence, the "whole foods" concept is being viewed as a better approach than a single dietary factor. It is believed that individual dietary factors in food may work additively or synergistically, to yield a better response in preventing diseases.

In modern times, the concept of multiagent therapeutics for cancer treatment has been in practice since the 1960s, with evidence of enhanced survival in childhood leukemias and Hodgkin's disease following combination chemotherapy (compared to a single agent).[32] Currently, most cancer chemotherapeutic drugs are used in combination in order to increase efficacy and/or decrease toxicity. The rationale for recommending a multidrug regimen is to attack more than one critical function in the cancer cells, leading to improved clinical outcomes. Thus, from ancient times to the modern era, combinatorial therapeutic strategies for disease management have been proven to be more efficacious than monotherapies. Based on recent studies and strong rationale, combination chemoprevention is being appreciated and investigated as a viable and effective strategy for cancer management.

Resveratrol-based combinations for cancer management

Based on encouraging recent research in a wide range of scientific disciplines, including cancer, heart diseases, metabolic conditions, and aging, resveratrol is probably the most extensively studied flavonoid at present. Recently, researchers began to focus on using resveratrol in conjunction with other agents and drugs for improved response against cancer. A few examples of recent research efforts on resveratrol-based combinatorial strategies

are discussed below. In this review, we have mainly focused on *in vivo* studies conducted in animal models. Table 1 provides a summary of *in vivo* studies where resveratrol-based combinations have been evaluated.

Resveratrol and piperine

A group of researchers believe that the biggest hurdle in the development of resveratrol as a drug or preventive agent is its poor bioavailability following oral ingestion, due to its rapid metabolism, mainly to its glucuronide and sulfate metabolites. We have recently reviewed this area of research and the different possibilities in this direction.[33] We believe that more research is needed to determine the possibility of chemopreventive efficacy of resveratrol metabolites as well as the possibility of obtaining and maintaining steady and effective *in vivo* resveratrol concentrations following chronic ingestion. However, researchers have begun to focus on different means of enhancing the bioavailability of resveratrol, as well as developing novel resveratrol analogues with superior efficacy and bioavailability. A recent study from our laboratory has shown that piperine, an alkaloid present in black pepper, can significantly enhance resveratrol levels in the blood of mice.[34] In this study, we found that addition of piperine significantly enhances the degree of exposure (i.e., AUC) to resveratrol as well as its maximum serum concentration (C_{max}) in C57BL mice.[34] Piperine has previously been shown to enhance the bioavailability of other polyphenols such as (−)-epigallocatechin-3-gallate.[35] In another interesting recent *in vitro* study, a resveratrol and piperine combination was found to act as a sensitizer for ionizing radiation-induced apoptotic cell death.[5] Although these studies are encouraging, the effect of piperine on resveratrol bioavailability remains unknown in the human population. Further, the therapeutic efficacy of this combination in disease models needs to be assessed.

Resveratrol and quercetin

Both resveratrol and quercetin are polyphenols present in red grapes, red wine, and several other plants. However, the levels of quercetin in red wine are typically ∼10-fold higher than resveratrol.[36] In a recent study, Khandelwal *et al.* have shown that resveratrol and quercetin synergistically reduce the extent of restenosis (a critical complication of angioplasty and stenting), possibly by inhibiting vascular smooth muscle cell proliferation and inflammation.[36] Further, in a study by Zhou *et al.*, transcriptomic and metabolomic profiling revealed the synergistic effects of quercetin and resveratrol supplementation in high-fat diet-fed mice.[37] It seems that additive/synergistic interactions between these two polyphenols may be one explanation for the "French Paradox," especially because both of these agents are present in red wine. Thus, the combination of resveratrol and quercetin seems to have potential toward cancer management. In addition, quercetin has also been shown to inhibit sulfation of resveratrol.[38] Therefore, it is conceivable that quercetin can enhance the bioavailability, and thus therapeutic efficacy, of resveratrol by inhibiting its sulfation. However, studies are needed to explore these possibilities.

Resveratrol and melatonin

Resveratrol has also been studied in combination with the pineal hormone and known antioxidant melatonin. Kiskova *et al.* have recently demonstrated that a combination of resveratrol with melatonin exerts superior chemopreventive effects in *N*-methyl-*N*-nitrosourea (NMU)-induced rat mammary carcinogenesis.[6] The data from this study showed that neither of the two agents alone had any appreciable effect on NMU-induced mammary carcinogenesis, the combination resulted in a significant decrease in tumor incidence. Further, another study found that melatonin synergistically enhanced resveratrol-induced heme oxygenase-1, possibly through inhibition of a ubiquitin-dependent proteasome pathway.[39] The authors suggested that this combination may provide an effective means to treat neurodegenerative disorders.[39] This combination seems to have potential in cancer chemoprevention. It is possible that these two agents may target two nonoverlapping pathways. Although melatonin can function through its own receptors, resveratrol may inhibit proliferative signaling by modulating other pathways. Thus, there is a possibility that this combination may lead to a synergistic response to attenuate proliferative signaling and improve cancer chemopreventive response.

Resveratrol and tea polyphenols

In a recent study, George *et al.* determined the effect of the combination of resveratrol with black tea polyphenol in a two-stage mouse skin carcinogenesis model. It was found that the combination

Table 1. Studies evaluating combinations of resveratrol with other agents

Agents used in combination with resveratrol	Model system	Outcome	References
Piperine	C57BL healthy mice	Piperine enhanced the serum bioavailability of resveratrol	34
Quercetin	Mice with a carotid injury	Combination synergistically reduced the extent of restenosis	36
Quercetin	High-fat diet-fed mice	Combination resulted in a restoration of high fat-induced alterations in pathways of glucose/lipid metabolism, liver function, cardiovascular system, and inflammation/immunity	37
Melatonin	NMU-induced rat mammary carcinogenesis	Combination resulted in a significant decrease in tumorigenesis	6
Black tea polyphenols	Two stage skin carcinogenesis mouse model	Combination resulted in a synergistic tumor suppressive response	7
Curcumin	BP-induced lung cancer in mice	Combination showed better chemopreventive response by maintaining adequate zinc, and modulating Cox-2 and p21 level	25
Quercetin + Genistein + Apigenin + EGCG + Baicalein + Curcumin	TRAMP mouse model of prostate cancer	All seven compounds inhibited well-differentiated carcinoma of the prostate by 58% when fed in combination as pure compounds; and 81% when fed as crude plant extracts	41
Geneistin	SV-40 rat model of prostate cancer	Combination reduced the most severe grade of prostate cancer in SV-40 Tag-targeted probasin promoter rat model	4
ProstaCaid	Nude mouse model of prostate cancer	ProstaCaid™, which contains a number of chemopreventive agents including resveratrol, inhibited invasive prostate cancer in a nude mouse model	42
Temozolomide	Nude mouse model of glioma	Resveratrol was found to enhance the therapeutic efficacy by inhibiting ROS/ERK-mediated autophagy and enhancing apoptosis	45
Doxorubicin (DOX)	B16/DOX mouse model of melanoma	Resveratrol was found to overcome chemoresistance by inducing cell cycle disruption and apoptosis	46
Quercitin + Catechin + Gefitinib	Nude mouse model of mammary cancer	Resveratrol, quercetin and catechin combination potentiated the effects of gefitinib in inhibiting mammary tumor growth	8

imparts a synergistic tumor-suppressive response, compared to either of the agents alone.[7] The authors suggested that the observed synergistic response is possibly due to a synergistic action of the two agents on same molecular targets. This is an interesting study because a synergistic action of multiple agents on a common pathway(s) can lead to dose-reduction of chemopreventive agents, thereby limiting the chances of side effects.

Resveratrol and curcumin

In a recent study, Malhotra *et al.* assessed the efficacy of combined supplementation of curcumin and resveratrol in benzo[a]pyrene (BP)-induced lung carcinogenesis in mice.[25] The study demonstrated that curcumin and resveratrol in combination provide a better chemopreventive response by maintaining adequate zinc levels and by modulating Cox-2 and p21.[25] Here, it is important to mention another study by Zhang *et al.,* which demonstrated that a combination of resveratrol and zinc in normal human prostate epithelial cells increased total cellular zinc and intracellular free labile zinc in the cells.[40] Since zinc is an extremely important trace element in normal prostate development as well as in prostate cancer, this finding provides a rationale to conduct further studies to evaluate the combination of zinc and resveratrol in prevention as well as treatment of prostate cancer.

Combination with other natural agents

A few other combinations containing resveratrol have also been investigated for their cancer chemopreventive effects in *in vivo* models. Slusarz *et al.* determined the preventive and therapeutic abilities of a number of agents along with resveratrol (quercetin, genistein, apigenin, baicalein, curcumin, and epigallocatechin 3-gallate (EGCG)), *in vitro* as well as *in vivo* in transgenic adenocarcinoma of the mouse prostate (TRAMP).[41] The authors found that four of the seven compounds (genistein, curcumin, EGCG, and resveratrol) inhibited Hedgehog signaling as shown by real-time reverse transcription-PCR analysis of Gli1 mRNA concentration or by Gli reporter activity.[41] The authors also found that all the seven compounds, when fed in combination as pure compounds or as crude plant extracts, inhibited well-differentiated carcinoma of the prostate by 58% and 81%, respectively. In another study, resveratrol in combination with genistein, provided in the diet, was found to significantly reduce the most severe grade of prostate cancer in the Simian Virus-40 T-antigen (SV-40 Tag)-targeted probasin promoter rat model, a transgenic model of spontaneously developing prostate cancer.[4] In another study, Jiang *et al.* have shown the anticancer efficacy of the dietary supplement ProstaCaid^{TM}, which contains a number of chemopreventive agents including resveratrol, against invasive prostate cancer in a nude mouse model.[42]

Resveratrol in combination with anticancer drugs

Plenty of *in vitro* and limited *in vivo* studies have suggested that resveratrol may enhance the antitumor effects of chemotherapeutic drugs in several cancers.[43,44] Thus, in addition to chemopreventive and cytostatic properties, resveratrol is being investigated for its potential as an adjuvant in conjunction with chemotherapeutic modalities to enhance their efficacy and/or limit their toxicities. Lin *et al.* have shown that resveratrol potentiated the therapeutic efficacy of temozolomide, an alkylating agent used in cancer therapeutics, in a mouse xenograft model of malignant glioma, through inhibiting ROS/ERK mediated autophagy and enhancing apoptosis.[45] Resveratrol has also been shown to overcome chemoresistance in a mouse model of B16/DOX melanoma by inducing cell cycle disruption and apoptosis, leading to reduced growth of melanoma and prolonged survival of mice.[46] In a recent study, a combination of the dietary grape polyphenols resveratrol, quercetin, and catechin was shown to potentiate the effects of gefitinib in inhibiting mammary tumor growth and metastasis in nude mice.[8] These studies support the potential use of resveratrol as an adjuvant in combination with chemotherapeutic drugs for cancer management. However, a study by Fukui *et al.* suggested that resveratrol may diminish the antiproliferative effect of paclitaxel in breast cancer.[47] Therefore, more preclinical studies in appropriate model are warranted to ascertain the usefulness of resveratrol as an adjuvant.

Resveratrol in combination with other factors within its natural matrix

As discussed above, emerging evidence suggests that the whole foods concept could be a better approach than single agents due to the possibility of synergistic improvement of responses from interactions between different ingredients within a food source.

For example, grapes contain several hundreds of ingredients with health-promoting properties. These individual agents may enhance the effectiveness and bioavailability of each other. Careful studies are needed to understand and to define whether an agent(s) should be considered in isolation, in combination, or in its natural complex form. A few examples of resveratrol-based naturally occurring combinations are provided below.

Crude extract of *Polygonum cuspidatum*, in addition to resveratrol, contains piceid (a glucoside precursor of resveratrol), polydatin (a stilbene), and emodin (an anthraquinone), among several other ingredients. All of these agents are considered as potential bioactive agents with health-promoting effects. A study by Ghamin *et al.* assessed the effect of a *Polygonum cuspidatum* extract (PCE) containing resveratrol on oxidative and inflammatory stress in healthy volunteers. Based on the data, the authors suggested that the PCE containing resveratrol had a comprehensive suppressive effect on oxidative and inflammatory stress.[48] In another phase I pilot study in colorectal cancer patients, Nguyen *et al.* found that resveratrol-containing freeze-dried grape powder inhibits the Wnt pathway, which is a key signaling pathway in colon cancer initiation; however, the effect was confined to the normal colonic mucosa.[49] Ortuno *et al.* conducted a pharmacokinetic study of resveratrol, in different matrices, in eleven healthy volunteers. The authors found that resveratrol was better absorbed from natural grape products than from supplements.[50] All of this evidence suggested that naturally available combinations of resveratrol and matrix of the source may be extremely important to the overall bioavailability and efficacy of resveratrol. This seems to be very important in cancer prevention settings, where chronic administration of resveratrol-containing moieties can possibly lead to an effective concentration of resveratrol *in vivo* to provide a chemopreventive response.

Conclusions

Based on emerging evidence, it is becoming increasingly clear that combination chemoprevention, relying on a combination of agents with limited (nonoverlapping) toxicity, which may diminish the toxicity of each while enhancing therapeutic efficacy, could be a better strategy for cancer management. Resveratrol is being extensively studied for chemoprevention in a variety of cancers. It appears that resveratrol possesses a number of characteristics of an ideal chemopreventive agent, such as (1) a lack of toxicity at desired concentrations, (2) available knowledge of mechanism(s) of action, (3) human acceptability because of being a dietary ingredient, and (4) cost affordability. Recent research is focusing on resveratrol-based combinatorial strategies for the management of cancer. As discussed before and depicted in Figure 1, resveratrol-based combinations can lead to improved chemopreventive and therapeutic response in a number of ways. On one hand, resveratrol may be used in combination with other naturally occurring chemopreventive agents in a cancer prevention setting. On the other hand, resveratrol may be used in conjunction with existing therapeutic modalities to enhance their response

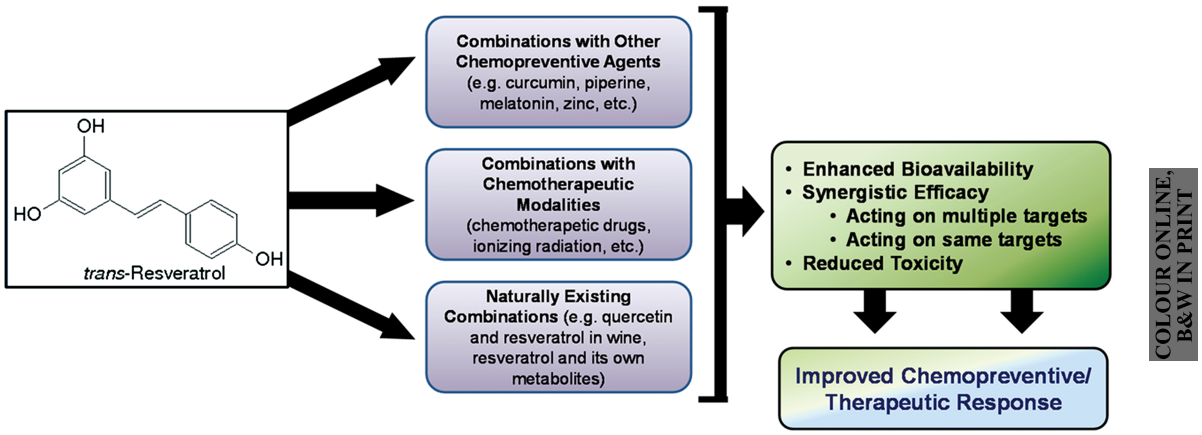

Figure 1. Resveratrol-based combinatorial strategies for cancer management.

and limit their toxicity. Indeed, further preclinical studies are required to define the most useful combinations. In addition, clinical studies are also needed to ascertain the efficacy of resveratrol in adjuvant settings.

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Conflict of interest

The authors declare no conflicts of interest.

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